Age, growth and reproduction of *Sarcocheilichthys nigripinnis* from the Qingyi Stream in the Huangshan Mountains

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Abstract: Identifying the life-history strategies of fish and their associations with the surrounding environment is the basic foundation in the conservation and sustainable utilization of fish species. We examined the age, growth, and reproduction of Sarcocheilichthys nigripinnis using 352 specimens collected monthly from May 2009 to April 2010 in the Qingyi Stream. We found the sex ratio of this study population was 0.58:1 (female: male), significantly different from expected 1:1. Females and males both comprised four age groups. The annuli on the scales were formed during February and March. No obvious between-sex difference was observed in length-weight and length-scale-radius relationships. The total length in back-calculation significantly increased with age for both sexes, but did not differ significantly at each age between the two sexes. An inflection point was observed in the growth curves given by the von Bertalanffy growth function for total weight. At this inflection point, fish were 3.95 years. Both sexes reach their 50% sex maturity at age 2, when females and males were 94.7 mm and 103.0 mm total length. The temporal pattern of the gonado-somatic index corresponded to a spawning period that occurred from April through July. The non-synchronicity of egg diameter in each mature ovary during the breeding period suggested these fish may be batch spawners. The absolute fecundity increased significantly with total length and weight, whereas no significant correlation was observed between the relative fecundity and body size.

Keywords: *Sarcocheilichthys nigripinnis*; Age structure; Annulus formation; Sexual maturity; Spawning period; Reproductive investment

The genus *Sarcocheilichthys* Bleeker (Cyprinidae, Gobioninae) is a group of small freshwater fish in East Asia, distributed from the Amur River basin to Song-Koi River in Northern Vietnam (Fujita et al, 2008; Zhang et al, 2008). These fish have an unusual mating system whereby the females lay eggs inside the mantle cavity of freshwater mussels by a prolonged oviduct, which is the same with most Rhodeinae (Cyprinidae) fish (Luo et al, 1977). At least 10 species and sub-species have been recorded for this genus, and the majority of which—approximately 8 species and sub-species—are located in China (Chen, 1998). The gudgeon *S. nigripinnis* is found in many areas of China, from the Yellow River basin to Hainan. This species is usually found in the benthic water of slow-flowing (the middle and lower reaches of

rivers) and lentic systems (lakes and reservoirs). They are omnivores mainly feeding on benthic invertebrates and alga (Chen, 1998). Although the phylogeny and speciation of this genus (Fujita et al, 2008; Zhang et al, 2008) and the biological traits of some species like *S. sinensis* (Guo et al, 1995; Song & Ma, 1994, 1996) and *S. variegates* (Yonezawa, 1958) have been documented, basic biological information of *S. nigripinnis* is rare.

Identifying the life-history traits, in particular growth and reproduction, of fish and their correlations with the surrounding environment is the foundation for developing sustainable utilization and conservation of these fishery resources (Yin, 1993). However, these fish often exhibit a myriad of life-history strategies among different taxonomic groupings and spatially separated populations of the same

species (Winemiller & Rose, 1992). The interspecific variation in life history results from the combined influences of environmental conditions and genetic traits (Stearns, 1992). The potential mechanisms explaining the intraspecific variation in life history involve environmental variation, sexual dimorphism, and genetic divergence (Hutchings et al, 2007; Yan & Chen, 2007; Young, 2005). In addition, life-history strategies are also constrained by the trade-off among these life-history traits (Stearns, 1992). As a result, taking account that the life-history traits of most stream fishes are still unknown, it is clearly necessary to improve the research on fish life history to better understand how stream fishes correlate with surrounding environment and to improve the management actions and conservation programs (Wootton et al, 2000). In this study, the age, growth, and reproduction of *S. nigripinnis*─the basic elements for management action and conservation programs─were examined in the Qingyi Stream, a tributary at the lower reach of the Yangtze River.

MATERIALS AND METHODS

Study area and fish collection

The Qingyi Stream is in the northern part of the Huangshan Mountains of Anhui Province, China, and flows northeast toward its confluence with the lower reach of the Yangtze River. This stream is 309 km long at mainstem and covers 7 195 km^2 of watershed area. Because of the local subtropical monsoon climate, this stream is characterized by the asymmetries of seasonal temperature and precipitation, with the mean monthly air temperatures ranging from –2.1 in January to 27.5 ℃ in July, and an annual mean temperature of 17.8 ℃. The annual precipitation is close to 2 000 mm/year, approximately 80% of which comes during the spring and summer, as less than5% comes during the cold and dry winter (Yan et al, 2011).

We collected fish using electro-fishing at Chenjiadan (N118°24′, E30°33′) in the middle reach of the Qingyi Stream. Sampling was conducted monthly during the third week of each month, from May 2009 to April 2010. *S. nigripinnis* was sorted out and individually measured for total length (L, 0.1 mm), weighed (wetted weight; W, 0.1 g), and dissected to determine sex from the visual traits of the gonads. The gonad was removed, scored in six maturity stages following Yin (1993), and weighed (gonad weight; GW, 0.01 g). The fish was reweighed after removal of the inner organs (somatic weight; SW, 0.1 g). All specimens and mature ovaries at stages IV, at which ovaries were close to maturity and characterized by well-deposited vitellogenic granules and well-developed vascularization, were preserved in 8% neutralized formalin solution.

During each sampling, the local environmental conditions of the sampling site were described by six variables: elevation, wetted width, water depth, water temperature, dissolved oxygen, and current velocity. Elevation was determined by a global positioning system. Wetted width was measured along five transects, regularly spaced across the stream channel. Water depth, temperature, and dissolved oxygen were surveyed at four equal-interval points along each transect (JENCO 9010, USA). Velocity was taken at 60% of water depth at each point (FP111 Flow Probe, USA). The seasonal stability in habitat variability was estimated by the coefficient of variability (CV), a percentage and calculated from the mean and standard deviation (SD) as follows: CV=SD/Mean (Grossman et al, 1990).

Age and growth

Scales taken between the posterior end of the pectoral fin and the anterior end of the dorsal fin were used to determine age. Age x was recorded when (x–1) annuli were observed on the scales. Age estimation was independently performed by two investigators and accepted when the two readings were identical; otherwise, further determination was performed by a third person. The age identified by the third person was accepted if it was identical with one of the readings by the former two readers; if not, this fish was deleted in age determination.

The scale radius (R, 0.1 mm) was measured using the Photo Analysis System software, 2.01 (Zhu et al, 2002). The timing of annulus formation was estimated from the monthly changes in the marginal increment ratio (MIR) as $MIR=(R-r_{max})/(r_{max}-r_{max-1})$, where R is the scale radius and r_{max} is the length of the outermost annulus.

The L–W equation was estimated from $W = aL^b$ and the L–R equation from $L=a+bR$. To estimate the total length at age, the back-calculated length (BCL) was obtained using the Fraser-Lee equation (Duncan, 1980): $L_n=a+(L-a) R_n/R$, where L_n is the L at the formation of the n-th annulus, a is the intercept in the L–R linear function, and R_n is the scale radius of the n-th annulus. Growth curves were fitted to the back-calculated data using the von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938):$L_t=L_\infty(1-e^{-k\ (t-t0)})$, where L_t is the predicted length at age t, L_∞ is the mean theoretical maximum length, k is the growth rate parameter and t_0 is the theoretical age at zero length.

Reproduction

Maturity was defined as the length and age at which 50% individuals of each sex were matured. $L_{50\%}$ and $T_{50\%}$ were estimated by fitting the binomial maturity data to the logistic function: $P=1/[1+e^{-(a+bx)}]$, where P is the proportion of mature fish in each 10-mm size or each 1-year age intervals, x is total length or age. Length and age at maturity were expressed as –a/b. Breeding timing was determined following the monthly changes of the

gonad-somatic index (GSI), calculated from $GSI=100(GW/SW)\%$. Absolute fecundity (AF) was estimated in terms of the number of the oocytes with vitellinogenic granules from mature ovaries following the gravimetric method (Bagenal & Braum, 1978), and relative fecundity (RF) was calculated from $RF=AF/SW$.

Data analysis

A one-sample chi-square test was used to test the difference between the observed sex-ratio and expected ratio of 1:1. Analysis of covariance (ANCOVA) was performed to compare $L–W$ or $L–R$ relationships between the sexes. One-way analysis of variance (ANOVA) was used to test the variations in BCLs across age groups, MIRs across months and GSIs across months, respectively. Newman-Keuls test was used for *post-hoc* comparisons after ANOVA. An independent-samples *t*-test was used to compare BCL at each age between the two sexes. Statistical significance was determined at $P<0.05$.

RESULTS

Habitat conditions

Elevation of this sampling site was relatively low, approximately 72.0 m. The streambed was mainly composed by mid-size substrates. Wetted width was 47.00±3.68 (mean±*SD*) m, water depth was 0.95±0.30 m, and current velocity was 0.21±0.05 m/s, with their maximum values occurring in summer and the minimum in winter. Water temperature ranged from 4.5 (January) to 28.3 °C (July), with the mean of 17.9 °C. Dissolved oxygen was relatively abundant, approximately $8.39×10^{-6}$ in average. Based on their CVs, wetted width was the most stable factor across months (0.08 of CV) while water temperature was the most unstable (0.40). The other three variables were relatively stable (0.32, 0.24, and 0.23 for those of water depth, velocity, and dissolved oxygen, respectively).

Age

The annuli on the scales of *S. nigripinnis* were easily identified because they showed the general annuli characteristics on the scales of most cyprinid, an obvious incising track occurring at the annulus location. Of the 352 specimens of *S. nigripinnis* collected, 129 were females (63.0–125.0 mm *L*, 2.8–22.2 g *W*) and 223 were males (64.0–119.3 mm *L*, 2.4–17.4 g *W*). The sex ratio estimated from sex-identified fish was 0.58:1 (female:male), significantly different from expected 1:1 ratio (χ^2=12.78, $P<0.05$). In terms of the numbers of annuli on scales, the oldest females and males were both four years old. The relative abundance of each age group were 31.0% (age 1), 47.3% (age 2), 17.8% (age 3), and 3.9% (age 4) for females, and 23.8%, 51.1%, 21.5%, and 3.6% (age 1–4)

for males, respectively. The preponderant age groups were aged 1 and 2 for both sexes, their relative abundance being approximately 80% collectively.

MIRs varied significantly across 12 months (one-way ANOVA, F=13.31, $P<0.01$). A significant decline in GSI was observed from January to March and from July to August ($P<0.05$), while a significant increase was from March through June ($P<0.05$). Some individual MIRs close to zero value occurred during February and March. The monthly changes in MIRs showed that the annuli on scales were formed during February and March (Figure 1).
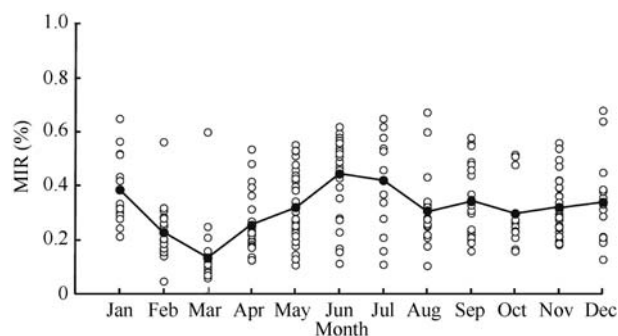

Figure 1   Monthly changes in the marginal increment ratio (MIR) for *S. nigripinnis* in the Qingyi Stream
Solid circles indicate the mean MIR.

Growth

The $L–W$ equation was $W=2.0×10^{-5}L^{2.89}$ (R^2=0.87, n=129) for females and $W=3.0×10^{-5}L^{2.80}$ (R^2=0.79, n=223) for males. These equations did not differ significantly between the sexes (ANCOVA, F=1.70, $P>0.05$). Accordingly, their $L–W$ relationships were combined to give $W=2.0×10^{-5}L^{2.85}$ (R^2=0.82, n=352). The $L–R$ equations for the females and males were both linear and were given by $L=0.02R+26.35$ (R^2=0.56, n=129) and $L=0.03R+34.94$ (R^2=0.60, n=223), respectively. They could be combined to give $L=0.02R+32.36$ (R^2=0.58, n=352) because of no significant difference in these equations between the two sexes (ANCOVA, F=0.50, $P>0.05$).

L_1, L_2, and L_3 in back-calculation were 80.3±10.8 mm, 90.4±13.5 mm, and 104.6±10.2 mm for females and 78.6±10.1 mm, 91.8±10.9 mm, and 97.9±12.6 for males, respectively. For both sexes, the BCLs were significantly different among age groups (one-way ANOVA, F=14.61, $P<0.05$ (females); F=35.58, $P<0.05$ (males)). Greater BCLs were observed at older ages. However, the BCLs at specific ages did not differ significantly between the two sexes (L_1 (*t*-test, F=0.32, $P>0.05$), L_2 (F=1.46, $P>0.05$), L_3 (F=0.36, $P>0.05$)).

Since the two sexes did not differ significantly in their $L–W$ and $L–R$ equations and their BCLs at each age, a combined VBGF fitting the back-calculated data was estimated collectively for both sexes as $L_t=172.3[1-e^{-0.14(t+3.53)}]$ and $W_t=47.2[1-e^{-0.14(t+3.53)}]^{2.85}$ (Figure 2).

Derived from the VBGFs in total length and weight, the growth rates were $dL/dt=24.12e^{-0.14(t+3.53)}$ and $dW/dt=18.84e^{-0.14(t+3.53)}[1-e^{-0.14(t+3.53)}]^{1.85}$, and the growth acceleration equations were $d^2Lt/dt^2=-3.38e^{-0.14(t+3.53)}$ and $d^2Wt/dt^2=2.64e^{-0.14(t+3.53)}[1-e^{-0.14(t+3.53)}]^{0.85}[2.85e^{-0.14(t+3.53)}-1]$ (Figure 3). As age increased, fish showed a decrease in the rate of growth in total length. However, an obvious inflection point was observed in the rate of growth in total weight at the age of 3.95 years (Figure 3).
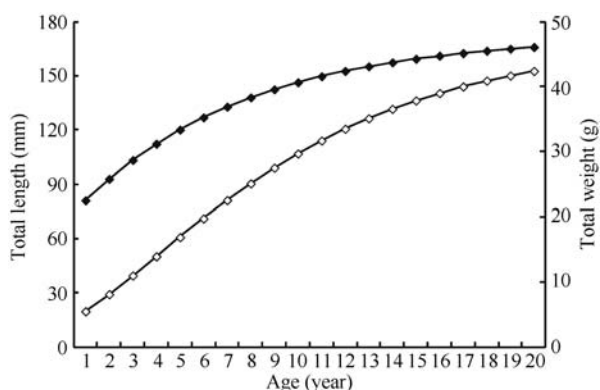

Figure 2 Von Bertalanffy growth function curves fitted to the length-at-age and weight-at-age data for S. nigripinnis in the Qingyi Stream
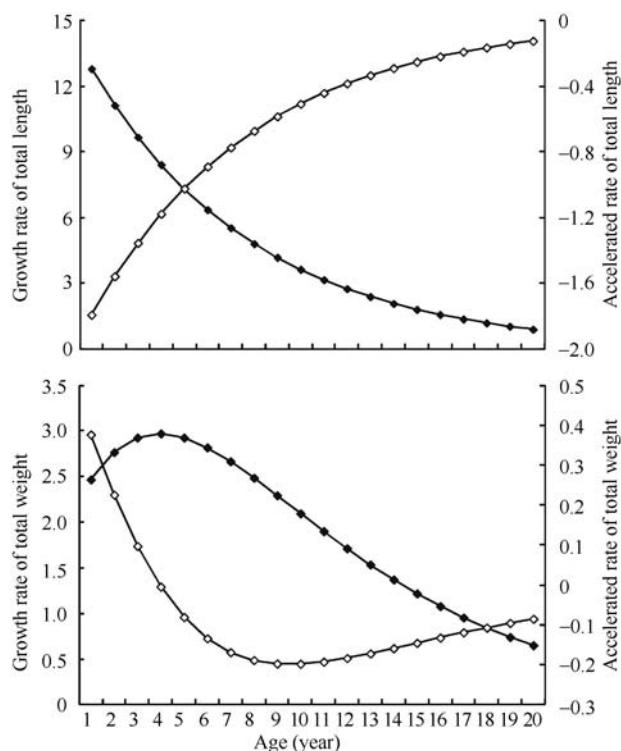Solid and dashed lines indicate length and weight, respectively.

Figure 3 Curves of growth rate and its acceleration in the von Bertalanffy growth function of length and weight for *S. nigripinnis* in the Qingyi Stream
Solid and dashed lines indicate growth rate and its acceleration, respectively.

Reproduction

Fifty percentages of the two sexes both reach sexual maturity at age 2, the second year following its birth. $L_{50\%}$ was 94.7 mm for females and 103.0 for males, respectively (Figure 4). The observed minimum total length at maturity was 73.0 mm for females and 90.9 mm for males.
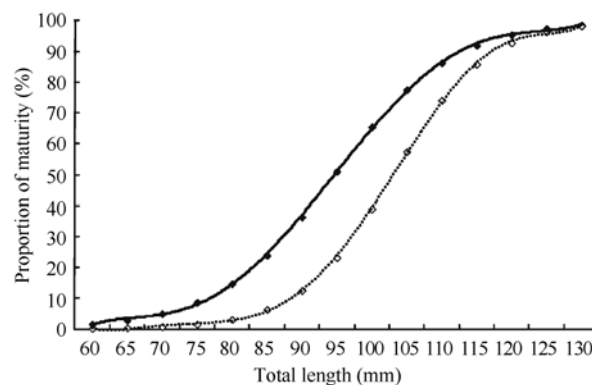

Figure 4 Percentage contributions of mature female and male *S. nigripinnis* in sequential 10-mm total-length intervals
Solid and dashed lines indicate the females and males, respectively.

Both sexes showed significant variations in GSI across the 12 months (one-way ANOVA, $F=45.02$, $P<0.01$ for females; $F=6.83$, $P<0.01$ for males). For females, the GSI increased markedly in February, remained relatively high (approximately 13.0%) from February through May, and then declined sharply during the period from June through August (Figure 5). The GSI of males did not vary acutely as that of females, but the two sexes showed a similarity in the temporal pattern in GSI (Figure 5). In addition, the gonads at stage V, when gonads were wholly matured, were observed during the period from April through July. Accordingly, the breeding activities of this species occur from April through July.
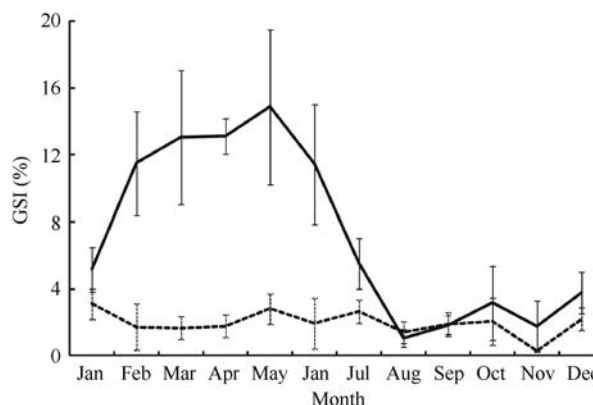

Figure 5 Monthly changes in gonado-somatic index (GSI) of female and male *S. nigripinnis* in the Qingyi Stream
Solid and dashed lines indicate the females and males, respectively.

The diameter of eggs from 10 mature ovaries in May did not significantly differ across the 10 ovaries (one-way ANOVA, F=0.12, P>0.05). However, the eggs of each ovary showed non-synchronicity in their developmental processes, because two obvious peaks were observed in the diameter-frequency distribution. The egg diameters ranged from 1.2 mm to 2.6 mm for the first peak and from 2.6 mm to 4.0 mm for the second peak, respectively (Figure 6). This suggests that *S. nigripinnis* in the Qingyi Stream are batch spawners.

A total of 32 mature females collected during April and May were used for fecundity estimation. AF ranged from 141 to 1 263 eggs, with a mean of 446 eggs. AF showed significantly correlations with total length (Pearson's correlation, P<0.01) and weight (P<0.01) and increased with total length and weight, as specified by the functions $AF=7.0\times10^{-5}L^{3.51}$ (R^2=0.41, n=32) and $AF=76.5W–83.4$ (R^2=0.49, n=32). RF ranged from 30.0 to 180.9 eggs/g, with a mean of 80.1 eggs/g, and was not significantly related with total length and weight (both P>0.05).
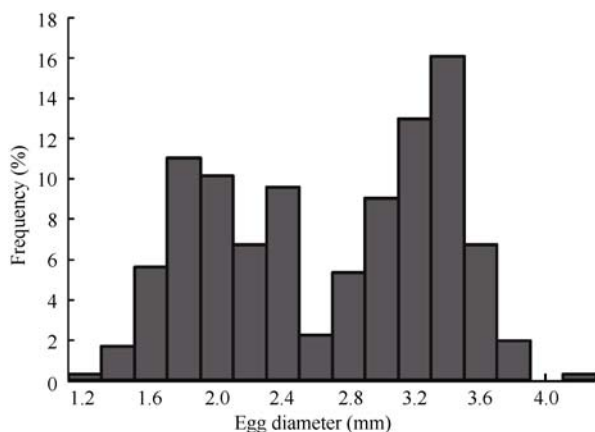

Figure 6 Frequency distribution of egg diameter in the breeding season for *S. nigripinnis* in the Qingyi Stream

DISCUSSION

Age structure and annulus formation

Based on the age determined from scales, both female and male *S. nigripinnis* in the Qingyi Stream comprised four age groups, with the preponderant age groups being ages 1 and 2 for both sexes, of which the relative abundance was approximately 80%. This distribution is similar to the age structure of another *Sarcocheilichthys* fish, *S. sinensis*, revealed by Song & Ma (1994) and Guo et al (1995), respectively. These authors found that the largest longevity of *S. sinensis* was four years in the middle reach of the Yangtze River (Song & Ma, 1994) and five years in the Xihe River in Sichuan Province, Western China (Guo et al, 1995). Additionally, our results showed that *S. nigripinnis* in the

Qingyi Stream reach the inflection point in weight-growth at the age of 3.95 years. The fact that most fish of *S. nigripinnis* are at a younger age than the inflection point suggests that this population has a young age structure. This age-lowering is also observed for other fish species like *Acrossocheilus fasciatus* (Yan et al, 2010a) and *Zacco platypus* (Xiang et al, 2009) in this study area. We suggest that it may be possible there are negative effects of local human activity, such as damming, over-fishing, and water pollution (Yan et al, 2009, 2010b, 2011) which play a role.

Our results on the monthly changes in MIR showed that the annuli on the scales of *S. nigripinnis* in the Qingyi Stream were formed during February and March. This result is approximately consistent with the timing of annuli formation for the other two cyprinid fish in the same stream, *A. fasciatus* (Yan et al, 2010a) and *Z. platypus* (Xiang et al, 2009). Annulus formation in fish is determined by either periodic environmental conditions (e.g., water temperature and food supply) or periodic biological events (e.g., reproduction and migration) (Lowe-McConnell, 1987; Yan & Chen, 2007). For riverine fish, the factors influencing annulus formation involve low water temperature, drawdown, and breeding activity of fish (Welcomme, 1979). Yan et al (2010a) and Xiang et al (2009) related the timing of annuli formation for the two cyprinid fish (*A. fasciatus* and *Z. platypus*, respectively) to the seasonal pattern of local water temperature in the Qingyi Stream. Locally low water temperature during the winter (approximately 0 °C) can constrain fish somatic growth, but the relatively high temperature during the late spring (approximately 20 °C) will release this constraint. Therefore, the resulting shift in fish somatic growth induces the annuli formation for fish during the late spring. A similar explanation may be suitable for the factor of drawdown, because rainfall varies seasonally with the similar rhythm with temperature in the Qingyi Stream watershed, characterized by the subtropical monsoon climate. However, due to our observation that *S. nigripinnis* spawn from April to July, the breeding activity of this population may not account for their annuli formation (Yan et al, 2010a).

Somatic growth and sexual dimorphism

In our results, the maximum total lengths of *S. nigripinnis* in the Qingyi Stream were 125.0 mm for females and 119.3 mm for males — lower than the historical maximum recorded total length for this species of approximately 140.0 mm (Chen, 1998). Taking no account of the possible difference in body size among different populations for this species, the difference in the maximum total lengths between this study and historical data perhaps suggests that this population in the Qingyi Stream has experienced the length-decreasing,

as well as the age-lowering discussed above. Potentially, over-fishing in this area may be an important cause for decreasing length of *S. nigripinnis,* because old fish with larger bodies are the primary target captured in fisheries (Yan et al, 2007). In addition, our results showed that the maximum total length in estimation was 172.3 mm for *S. nigripinnis* in the Qingyi Stream, which is higher than the recorded 140.0 mm. Despite of the general reasons that fish cannot reach their theoretical life-expectancy because of ecological limitation (e.g., abiotic factors, resources, predators, and disease) (Yin, 1993), the possible absence of old-fish data in this study may possibly result in the overestimation of the maximum theoretical total length of this species (Yin, 1993).

Sexual size dimorphism—the body size of the male relative to the body size of the female—reflects the relative influence of natural and sexual selections on both sexes (Shine, 1989). For many fish species, females are larger than males, which is favorable for females to enhance their reproductive investment and offspring output (Hedrick & Temeles, 1989). Alternatively, if natural selection for female fecundity is weaker than sexual selection, males are larger than females, resulting in mating systems with male-male contests and female choice for larger male size (Pyron, 1996). Our results showed that the two sexes of *S. nigripinnis* did not differ significantly in their *L–W* and *L–R* equations and their total length back-calculated at each age. This indicates that this species does not present sexual size dimorphism. However, associated with their unusual mating system where females lay eggs inside the mantle cavity of freshwater mussels, the female has a prolonged oviduct beneath its cloacal aperture during the breeding seasons (Luo et al, 1977), and the male is characterized by more florid body color than the female (Chen, 1998). Consequently, for *S. nigripinnis*, sexual selection is perhaps the primary driver determining the evolution in sexual dimorphism, and female fish are prone to choose the males with more florid body color, not larger body size, in their mating system with male-male contests. Furthermore, it is worth mentioning that our results showed that the females of *S. nigripinnis* were significantly fewer than males. This suggests the potentially intensive male-male contest in the mating system of this species, as most cyprinid fish are generally paired by one female and one male in their mating course, regardless of the fish population sex ratio (Yin, 1993).

Timing of spawning and sexual maturity

Most riverine fish spawn during the period coinciding with the timing of local flood (Welcomme, 1979). Alkins-Koo (2000) explained this flood-related adaptive spawning by arguing the mechanisms of flooding could extend the breeding habitat, increase food availability, and alleviate crowding and predation pressure. Our results showed that *S. nigripinnis* in the Qingyi Stream spawn from April to July. This timing is consistent with the observed spawning time for *S. sinensis* in the middle reach of the Yangtze River (Song &Ma, 1994), but different from *S. sinensis* in the Xihe Stream in Sichuan Province, which spawn from March through May (Guo et al, 1995). Additionally, for *A. fasciatus* (Yan et al, 2009) and *Z. platypus* (Xiang et al, 2009) in the Qingyi Stream, their breeding timings are approximately similar with the result we observed in this study. Due to the local subtropical monsoon climate, most annual precipitation occurs from April through September within the Qingyi watershed. Clearly, the timings of spawning of these fish in the Qingyi Stream coincide with local flooding season, suggesting the flood-related adaptive spawning for these fish.

Sexual maturity, an important life-history event of fish, is determined by genetic traits and influenced by environmental factors (Stearns, 1992). Trade-offs among different life-history variables also affect the age and size at sexual maturity. For example, early maturity could result in shorter longevity, slower somatic growth, and lower absolute fecundity (Heino et al, 2002). In our results, both female and male *S. nigripinnis* reach 50% maturity at the age of 2 years, which is consistent with those of *S. sinensis* in the middle reach of the Yangtze River (Song & Ma, 1994) and of the two small cyprinids, *A. fasciatus* (Yan et al, 2009) and *Z. platypus* (Xiang et al, 2009), in this study stream. This early maturity observed for these fish species, as well as short longevity, fast somatic growth, and low fecundity, is perhaps due to the life-history strategies of most small fish having opportunistic and periodic strategy (Winemiller, 1992).

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Zoological Research Call for papers

The 1st issue, 2013, "Special Issue for Primates and Animal Models of Human Diseases"

To whom it may concern:

Co-organized by *the Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province* and *Zoological Research*, two *special issues for Primates and Animal models of Human diseases* [32(1),2011 & 33(1),2012] have been released. The first special issue [32(1), 2011] has significantly increased the journal hits on PubMed (500 times more than last month) and during the next couple of months, the editorial office received about 40 related submissions. After peer-reviewing, 18 manuscripts have been accepted and published in the second special issue [33(1),2012].

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